

GENE ENCODING CYCLIC LIPOPEPTIDE ACYLASE AND
EXPRESSION OF THE SAME

Technical Field

5 The present invention relates to an enzyme that deacylates an acyl side chain of a cyclic lipopeptide substance (hereinafter to be also referred to as cyclic lipopeptide acylase), a gene encoding same, a method of producing cyclic lipopeptide acylase by genetic engineering
10 using the gene and a method of deacylating an acyl side chain of a cyclic lipopeptide substance.

Background Art

As an enzyme deacylating an acyl side chain of cyclic lipopeptide substances, such as FR901379 substance and analogs
15 thereof, there has been reported enzymes produced by the bacteria belonging to the genus *Streptomyces* (e.g., *Streptomyces anulatus* No. 4811 strain, *Streptomyces anulatus* No. 8703 strain, *Streptomyces* sp. No. 6907 strain) (WO97/32975). WO97/47738 further reports enzymes produced by
20 *Oidiodendron tenuissimum* IFO 6798 strain, *Oidiodendron echinulatum* IFO 31963 strain, *Oidiodendron truncatum* IFO 9951 strain, *Oidiodendron truncatum* IFO 31812 strain, *Oidiodendron* sp. No. 30084 strain and *Verticillium* sp. No. 30085 strain.

A large scale production and shortened production time of
25 these enzymes have been desired.

Disclosure Of The Invention

The present invention aims at harvesting cyclic lipopeptide acylase more efficiently. More particularly, the present invention aims at determining the amino acid sequence
30 of the cyclic lipopeptide acylase, determining a gene that encodes the same, and providing a method for producing the enzyme by genetic engineering using the gene and a method for deacylating an acyl side chain of a cyclic lipopeptide substance.

The present inventors have conducted intensive studies in an attempt to achieve the above-mentioned objects and succeeded in cloning the coding region of cyclic lipopeptide acylase, which resulted in the completion of the present invention. The present inventors have further introduced an expression vector containing the DNA into a host cell to allow expression of the activity of the cyclic lipopeptide acylase.

Accordingly, the present invention provides the following.

[1] A gene encoding cyclic lipopeptide acylase, which contains the entirety or a part of the following (a), (b) or (c):

(a) a DNA consisting of the nucleotide sequence depicted in SEQ ID No. 1

(b) a DNA capable of hybridizing with the DNA of the above-mentioned (a) under stringent conditions

(c) a DNA having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence depicted in SEQ ID No. 1.

[2] A gene encoding a protein of the following (a) or (b) or a part thereof:

(a) a protein consisting of the amino acid sequence depicted in SEQ ID No. 2

(b) a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in the amino acid sequence (a), which protein has a cyclic lipopeptide acylase activity.

[3] A recombinant vector containing the gene of the above-mentioned [1] or [2].

[4] An expression vector functionally containing the gene of the above-mentioned [1] or [2].

[5] A transformant obtained by transforming a host cell with the vector of the above-mentioned [3] or [4].

[6] A method of producing cyclic lipopeptide acylase, which comprises

culturing a host cell transformed with the expression vector of the above-mentioned [4], and harvesting, from the obtained culture, cyclic lipopeptide acylase capable of catalyzing a reaction to deacylate a side chain acylamino group of a cyclic lipopeptide substance into an amino group.

[7] A cyclic lipopeptide acylase produced by the production method of the above-mentioned [6].

[8] A gene encoding cyclic lipopeptide acylase, which contains the entirety or a part of the following (a), (b) or (c):

- (a) a DNA consisting of a nucleotide sequence shown by nucleotide No. 1065 to 3359 in the nucleotide sequence depicted in SEQ ID No. 1
- (b) a DNA capable of hybridizing with the DNA of the above-mentioned (a) under stringent conditions
- (c) a DNA having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence shown by nucleotide No. 1065 to 3359 in the nucleotide sequence depicted in SEQ ID No. 1.

[9] A gene encoding a protein of the following (a) or (b):

- (a) a protein consisting of amino acid number from -1 or 1 to 765 in the amino acid sequence depicted in SEQ ID No. 2
- (b) a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in the amino acid sequence (a), which protein has a cyclic lipopeptide acylase activity.

[10] A recombinant vector containing the gene of the above-mentioned [8] or [9].

[11] An expression vector functionally containing the gene of the above-mentioned [8] or [9].

[12] A transformant obtained by transforming a host cell with a vector of the above-mentioned [10] or [11].

[13] A method of producing cyclic lipopeptide acylase, which comprises

culturing a host cell transformed with the expression vector of the above-mentioned [11], and
5 harvesting, from the obtained culture, cyclic lipopeptide acylase capable of catalyzing a reaction to deacylate a side chain acylamino group of a cyclic lipopeptide substance into an amino group.

[14] A cyclic lipopeptide acylase produced by the production
10 method of the above-mentioned [13].

[15] A cyclic lipopeptide acylase encoded by a DNA consisting of a nucleotide sequence shown by nucleotide No. 1065 to 3359 in the nucleotide sequence depicted in SEQ ID No. 1.

[16] A cyclic lipopeptide acylase which is encoded by a DNA
15 having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence shown by nucleotide No. 1065 to 3359 in the nucleotide sequence depicted in SEQ ID No. 1.

[17] A protein of the following (a) or (b):

20 (a) a protein consisting of the amino acid number from -1 or 1 to 200 in the amino acid sequence depicted in SEQ ID No. 2

(b) a protein having an amino acid sequence involving deletion, substitution or addition of one to several
25 amino acid(s) in the amino acid sequence (a), which protein forms a complex with the protein of the following [18] to show a cyclic lipopeptide acylase activity.

[18] A protein of the following (c) or (d):

30 (c) a protein consisting of the amino acid No. 201 to 765 in the amino acid sequence depicted in SEQ ID No. 2

(d) a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in the above-mentioned amino acid

sequence (c), which protein forms a complex with the protein of the above-mentioned [17] to show a cyclic lipopeptide acylase activity.

[19] A DNA encoding the protein of the above-mentioned [17].

5 [20] A DNA encoding the protein of the above-mentioned [18].

[21] A recombinant vector containing at least one of the above-mentioned [19] and [20].

[22] An expression vector containing at least one of the above-mentioned [19] and [20].

10 [23] A transformant obtained by transforming a host cell with the vector of the above-mentioned [21] or [22].

[24] A method of producing cyclic lipopeptide acylase, which comprises

15 culturing a host cell transformed with the expression vector of the above-mentioned [22], and
harvesting, from the obtained culture, cyclic lipopeptide acylase capable of catalyzing a reaction to deacylate a side chain acylamino group of a cyclic lipopeptide substance into an amino group.

20 [25] A cyclic lipopeptide acylase produced by the production method of the above-mentioned [24].

[26] A method for deacylating a side chain acylamino group of a cyclic lipopeptide substance into an amino group, which method comprising culturing a host cell transformed with the
25 expression vector of the above-mentioned [4], [11] or [22], and bringing the cyclic lipopeptide substance into contact with the obtained culture or a treated product thereof.

Brief Description Of The Drawings

Fig. 1 shows a determined N-terminal amino acid sequence
30 of part of the cyclic lipopeptide acylase small subunit.

Fig. 2 shows a determined N-terminal amino acid sequence of part of the cyclic lipopeptide acylase large subunit.

Fig. 3 shows a gene map of *Streptomyces lividans* 1326/pIJ702-SB.

Detailed Description Of The Invention

In the present invention, cyclic lipopeptide acylase is an enzyme that deacylates an acyl side chain of, for example, FR901379 substance, an analog thereof and a relative, such as
5 echinocandin B.

The cyclic lipopeptide acylase of the present invention consists of two, large and small, subunits, and each subunit has the following characteristics. Each subunit forms a complex, and shows the cyclic lipopeptide acylase activity.

10 Large subunit:

(i) Molecular weight: about 61 kDa (SDS-PAGE)

(ii) Amino acid analysis:

The N-terminal amino acid sequence is Ser-Asn-Ala-Val-Ala-Phe-Asp-Gly-Ser-Thr-Thr-Val-Asn-Gly-Arg-Gly-Leu-Leu-Leu-
15 Gly (SEQ ID No. 3) or an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in said amino acid sequence.

(iii) A protein encoded by a DNA consisting of a nucleotide sequence shown by nucleotide No. 1665 to 3359 in the
20 nucleotide sequence depicted in SEQ ID No. 1, a DNA capable of hybridizing with this DNA under stringent conditions, or a DNA having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence.

25 (iv) A protein consisting of an amino acid sequence shown by amino acid No. 201 to 765 in the amino acid sequence depicted in the SEQ ID No. 2 or a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in said amino acid sequence, which protein forms
30 a complex with the following small subunit to show a cyclic lipopeptide acylase activity.

Small subunit:

(i) Molecular weight: about 19 kDa (SDS-PAGE)

(ii) Amino acid analysis:

The N-terminal amino acid sequence is Gly-Ser-Gly-Leu-Ser-Ala-Val-Ile-Arg-Tyr-Thr-Glu-Tyr-Gly-Ile-Pro-His-Ile-Val-Ala (SEQ ID No.4) or an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in
5 said amino acid sequence.

(iii) A protein encoded by a DNA consisting of a nucleotide sequence shown by nucleotide No. 1065 to 1664 in the nucleotide sequence depicted in SEQ ID No. 1, a DNA capable of hybridizing with this DNA under stringent conditions, or a DNA
10 having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence.

(iv) A protein consisting of an amino acid sequence shown by amino acid number from -1 or 1 to 200 in the amino acid
15 sequence depicted in SEQ ID No. 2 or a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in said amino acid sequence, which protein forms a complex with the above-mentioned large subunit to show a cyclic lipopeptide acylase activity.

20 The cyclic lipopeptide acylase of the present invention is not particularly limited as to its derivation, as long as it shows the above-mentioned characteristics, and includes one derived from an organism naturally present, a natural or artificial mutant, a variant, or one derived from a
25 transformant obtained by introducing a foreign cyclic lipopeptide acylase gene.

A method for artificially producing a mutant may be, for example, site-specific mutagenesis. More specifically, by introducing optional mutation into the nucleotide sequence
30 depicted in SEQ ID No. 1 by this method, a mutant cyclic lipopeptide acylase can be obtained. The thus-obtained mutant cyclic lipopeptide acylase has the above-mentioned characteristics.

The cyclic lipopeptide acylase of the present invention

can be obtained by suitably using a known method such as (1) a method including isolation and purification of a culture of a cell or tissue producing the enzyme as a starting material, (2) a method including chemical synthesis, (3) a method including purification from a cell processed by a genetic recombination technique and the like to express cyclic lipopeptide acylase, and the like.

For example, the cyclic lipopeptide acylase of the present invention can be isolated and purified as follows.

That is, a cell expressing cyclic lipopeptide acylase is cultured in a suitable liquid medium, and extracted and purified by a known method from the obtained culture. The method for the extraction and purification is a known suitable method for the fraction containing the objective product.

The method specifically includes the following. According to a conventional method, a culture is filtrated as it is or treated by centrifugation and the like to recover a cell or a supernatant. When an enzyme is accumulated in the cell, the recovered cell is suspended in a suitable buffer and a detergent is added at a suitable concentration to solubilize the membrane. When a host cell has a cell wall, a pretreatment with a lysozyme or ultrasonication is necessary. The detergent may be, for example, sodium dodecyl sulfate (SDS), cetyltrimethylammonium bromide (CTAB) and the like, but since these have a strong protein denaturing action, a gentle nonionic detergent, such as Triton X-100 and the like, is preferably used to make a protein folded to have a biological activity. Then, the obtained crude extract solution is processed according to a suitable combination of the methods generally employed in the presence of a detergent, where necessary, to isolate and purify the enzyme.

When the enzyme is present in a culture medium, the culture is filtrated or centrifuged to remove sediment (solid such as cell and the like), leaving only the solution, which

is processed according to a suitable combination of the methods generally employed to isolate and purify the objective substance. Examples of the method include a method utilizing solubility, such as salting out, solvent precipitation and the like, a method utilizing difference in molecular weights, such as dialysis, ultrafiltration, gel filtration, SDS-PAGE and the like, a method utilizing charge, such as ion exchange chromatography and the like, a method utilizing specific affinity, such as affinity chromatography and the like, a method utilizing difference in hydrophobicity, such as reverse phase high performance liquid chromatography and the like, a method utilizing difference in isoelectric points, such as isoelectric focusing method and the like, and the like. More specifically, separation and purification can be achieved by a conventional method, such as vacuum concentration, lyophilization, extraction with conventional solvent, pH adjustment, treatments using conventional adsorbent (e.g., anion exchange resin or cation exchange resin, nonionic exchange resin etc.), crystallization, recrystallization and the like. The detail is shown in the method described in WO97/32975.

The production of the cyclic lipopeptide acylase of the present invention by chemical synthesis includes using, for example, the nucleotide sequence depicted in SEQ ID No. 1, and specifying the amino acid encoding the entirety or a part of the sequence, and synthesizing or semi-synthesizing the amino acid using a peptide synthesizer.

The cyclic lipopeptide acylase gene is generally cloned by the following method. First, the enzyme is completely or partially purified by a conventional method from a cell or tissue that produces the cyclic lipopeptide acylase and the N-terminal amino acid sequences of the large subunit and small subunit are determined by the Edman method. Each subunit is partially degraded with a sequence-specific protease and the

amino acid sequence of the obtained oligopeptide is similarly determined by the Edman method. The oligonucleotide having a nucleotide sequence corresponding to the identified partial amino acid sequence is synthesized, and using this as a primer
5 or probe, each subunit is cloned from RNA or DNA prepared from a cell or tissue producing the cyclic lipopeptide acylase by the PCR method or colony (or plaque) hybridization.

Based on the obtained nucleotide sequence of each subunit, oligonucleotide is synthesized, and using this as a primer,
10 cyclic lipopeptide acylase is again cloned from RNA or DNA prepared from a cell or tissue producing the cyclic lipopeptide acylase by the PCR method or colony (or plaque) hybridization.

Alternatively, it is also possible to clone a DNA
15 encoding the enzyme and/or a subunit of the enzyme by preparing an antibody against the enzyme or a subunit thereof according to a conventional method using the entirety or a part of completely or partially purified cyclic lipopeptide acylase as an antigen, and screening using the antibody from
20 cDNA or genomic DNA library prepared from a cell or tissue producing the cyclic lipopeptide acylase.

Independently from the aforementioned method, moreover, a DNA encoding the cyclic lipopeptide acylase of the present invention can be directly cloned by the PCR method. That is, a
25 DNA fragment containing a coding region of a cyclic lipopeptide acylase, a coding region of a large subunit or a coding region of a small subunit can be amplified by PCR according to a conventional method using a genomic DNA or cDNA (or mRNA) derived from a cell or tissue having the enzyme
30 activity as a template and using, as a primer, a pair of suitable oligonucleotides, wherein an amplified fragment covers a coding region of a cyclic lipopeptide acylase, a coding region of a large subunit or a coding region of a small subunit.

The nucleotide sequence of the obtained DNA insert can be determined by a known sequencing technique such as Maxam-Gilbert method, dideoxy termination method and the like.

The gene encoding cyclic lipopeptide acylase of the present invention includes the entirety or a part of a DNA substantially consisting of a nucleotide sequence depicted in SEQ ID No. 1. As used herein, by the "DNA substantially consisting of" is meant a DNA having the above-mentioned specific nucleotide sequence, a DNA having a nucleotide sequence capable of hybridizing with the above-mentioned DNA having the specific nucleotide sequence under stringent conditions (in the present invention, the conditions under which a DNA having nucleotide sequence having not less than about 60% homology can hybridize, where the stringency can be adjusted by changing the temperature, salt concentration and the like during hybridization, and washing as appropriate), or a DNA having at least (1) 60% identity, (2) 70% identity, (3) 80% identity, (4) 90% identity or (5) 95% identity with the nucleotide sequence depicted in SEQ ID No. 1. The cyclic lipopeptide acylase that the gene codes for can deacylate an acyl side chain of FR901379 substance, an analog thereof and a relative thereof, such as echinocandin B, where each subunit has the aforementioned physicochemical properties.

Another embodiment of the gene encoding cyclic lipopeptide acylase of the present invention includes the entirety or a part of a DNA substantially consisting of a nucleotide sequence shown by nucleotide No. 1065 to 3359 in the nucleotide sequence depicted in SEQ ID No. 1. As used herein, the "DNA substantially consisting of" means as defined above.

Moreover, the present invention provides a gene encoding a protein of the following (a) or (b):

(a) a protein consisting of amino acid number from -1 or 1 to 765 in the amino acid sequence depicted in SEQ ID No. 2

(b) a protein having an amino acid sequence involving deletion, substitution or addition of one to several amino acid(s) in the amino acid sequence (a), which protein has a cyclic lipopeptide acylase activity.

5 The present invention also provides a gene encoding a large subunit and a small subunit of the above-mentioned cyclic lipopeptide acylase.

 The gene of the present invention may be obtained by any method. For example, the gene includes a complementary DNA
10 (cDNA) prepared from mRNA, a genomic DNA prepared from genomic library, a chemically synthesized DNA, a DNA obtained by amplifying by PCR using RNA or DNA as a template, a DNA constructed by a suitable combination of these methods and the like.

15 The present invention also relates to a recombinant vector containing any of the above-mentioned genes. The recombinant vector of the present invention is not particularly limited as long as it can be retained by replication or autonomous growth in various host cells, such
20 as prokaryocyte and/or eucaryocyte, and encompasses a plasmid vector, a phage vector and the like. The recombinant vector can be conveniently prepared by inserting any of the above-mentioned DNAs into a cloning vector or expression vector available in this field, utilizing a suitable restriction
25 enzyme site.

 Particularly, the recombinant vector of the present invention is an expression vector functionally containing a gene encoding cyclic lipopeptide acylase. As used herein, by the "functionally" is meant that a gene (DNA) is located such
30 that the gene is transcribed in a host cell suitable for the vector and the protein encoded by the gene can be produced. Preferably, it is a vector containing an expression cassette wherein a promoter region, an initiation codon and a gene encoding cyclic lipopeptide acylase or each subunit thereof, a

termination codon and a terminator region are sequentially arranged. The expression vector to be used is not particularly limited as long as it contains a promoter region that functions in various host cells of prokaryocyte and/or
5 eucaryocyte and expresses the gene downstream thereof and a signal that terminates the transcription of the gene, namely, a terminator region, and the promoter region and the terminator region are connected via a sequence containing at least one unique restriction enzyme recognition site. It is
10 preferable that it further contain a selection marker gene for selection of a transformant. Where desired, the expression vector may contain an initiation codon and a termination codon downstream of the promoter region and upstream of the terminator region, respectively.

15 When an expression vector containing a gene encoding a large subunit (or small subunit) of cyclic lipopeptide acylase is used for the production of cyclic lipopeptide acylase, an expression vector containing, in addition to the DNA, a DNA encoding a small subunit (or large subunit) in a state that
20 affords expression in a host cell is used. The DNA encoding a large subunit and a DNA encoding a small subunit may be placed under the control of different promoters or placed in tandem under the control of the same promoter.

For secretion of cyclic lipopeptide acylase into a medium
25 to obtain the acylase, the expression vector of the present invention preferably contains functionally the signal sequence. The signal sequence is not particularly limited as long as the protein secretion mechanism of the host cell can be recognized. When the host cell is *Actinomycetes*, those belonging to the
30 genus *Streptomyces* are preferable, which are derived from the same origin as the gene encoding cyclic lipopeptide acylase of the present invention. The signal sequence is cleaved and removed by protease in the cell and mature protein is secreted out of the cell.

When bacteria are used as a host cell, an expression vector generally needs to contain, in addition to the above-mentioned promoter region and terminator region, a replicatable unit that can autonomously replicate in a host cell. A promoter region contains a promoter, an operator and a Shine-Dalgarno (SD) sequence. For example, when the host is *E. coli*, Trp promoter, lac promoter, recA promoter, lpp promoter, tac promoter and the like are used as the promoter region, when the host is *Bacillus subtilis*, SP01 promoter, SP02 promoter, penP promoter and the like are used as the promoter region and when the host is *Actinomycetes*, melC, tipA, ermE, aphI and the like are used. As the terminator region, conventionally used natural or synthetic terminators can be used. As the selection marker gene, genes resistant to various drugs such as tetracycline, ampicillin, kanamycin, thiostrepton and the like can be used. As the initiation codon, ATG is generally used, but GTG may be also used. As the termination codon, conventional TGA, TAA and TAG can be used.

When the gene encoding cyclic lipopeptide acylase of the present invention is prepared from a genomic DNA derived from a cell or tissue producing the enzyme and is obtained in the form containing the original promoter and terminator regions, the expression vector of the present invention can be prepared by inserting the DNA into a suitable site of a known cloning vector capable of replicatable retention or autonomous growth in a host cell to be transformed. The cloning vector to be used when the host is bacteria includes pBR vector, pUC vector and the like derived from *E. coli*, pUB110, pTP5, pC194 and the like derived from *Bacillus subtilis*, pIJ702, pSK1, pSK2, SCP2, SCP1.2, pGA482, pMCXpress and the like derived from *Actinomycetes*.

The transformant of the present invention can be prepared by transforming a host cell with a recombinant vector containing a gene encoding cyclic lipopeptide acylase of the

present invention. The host cell is not particularly limited as long as it is compatible with the recombinant vector to be used and can be transformed. Various cells generally used in this field, such as naturally occurring cell, artificially
5 prepared mutant cell, recombinant cell and the like can be used. It is preferably bacteria, particularly *E. coli*, *Bacillus subtilis* and *Actinomycetes* and the like, and more preferably bacteria belonging to the genus *Streptomyces*, which is one kind of *Actinomycetes*.

10 A recombinant vector can be introduced into a host cell by a conventionally known method. For example, when the host cell is *E. coli*, *Bacillus subtilis* and the like, the method of Cohen et al. [Proc. Natl. Acad. Sci. USA, 69: 2110 (1972)], protoplast method [Mol. Gen. Genet., 168: 111 (1979)],
15 competent method [J. Mol. Biol., 56: 209 (1971)] and the like can be used.

When the host is *Actinomycetes*, particularly bacteria belonging to the genus *Streptomyces*, a method (PEG-assisted protoplast transformation) described in "Transformation
20 Genetic Manipulation of *Streptomyces*. A Laboratory Manual. The John Innes Foundation, Norwich, UK, 1985" and the like can be used.

The present invention also provides cyclic lipopeptide acylase.

25 The cyclic lipopeptide acylase of the present invention can be produced by culturing any of the transformants containing an expression vector functionally containing the above-mentioned gene encoding cyclic lipopeptide acylase in a suitable medium and harvesting the enzyme from the obtained
30 culture. The isolation and purification can be performed by suitably combining various separation techniques generally used as mentioned above according to the fraction showing a cyclic lipopeptide acylase activity. When produced by each subunit unit, an expression vector functionally containing the

gene encoding each subunit is used for similar production.

The nutrient medium to be used contains, as sources of carbon, carbohydrates such as glucose, xylose, galactose, glycerine, starch, dextrin and the like, and as other sources
5 of carbon, maltose, rhamnose, raffinose, arabinose, mannose, salicin, sodium succinate, fructose, mannitol, glucitol, lactose, sorbose, sucrose and the like can be used.

The preferred sources of nitrogen may include inorganic and organic nitrogen sources such as ammonium sulfate,
10 ammonium chloride, casein hydrolysate, yeast extract, polypeptone, bactotrypton, beef extract, soybean powder, wheat germ, potetoprotein, rice bran, peanut powder, gluten, corn extract and the like. Where desired, other nutrient sources [e.g., inorganic salt (e.g., sodium diphosphate, potassium
15 diphosphate, potassium phosphate, magnesium chloride, magnesium sulfate, calcium chloride), vitamins (e.g., vitamin B1), antibiotics (e.g., ampicillin, kanamycin, thiostrepton) and the like] may be added to a medium.

If necessary, especially when the culture medium foams
20 seriously a defoaming agent, such as liquid paraffin, fatty oil, plant oil or silicone, or the like may be added.

A transformant can be cultured generally at pH 4 - 9, preferably 6 - 7, at 15 - 35°C, preferably 25 - 35°C for 10 - 144 hours.

25 According to the method for deacylating a side chain acyl group of a cyclic lipopeptide substance of the present invention, any of the above-mentioned transformants containing an expression vector functionally containing a DNA encoding cyclic lipopeptide acylase is cultured in a suitable medium,
30 and using the obtained culture as it is, cyclic lipopeptide substance is brought into contact therewith to deacylate a side chain acyl group of the substance into an amino group. Alternatively, when the enzyme activity is found in the intracellular fraction of the transformant, by bringing cyclic

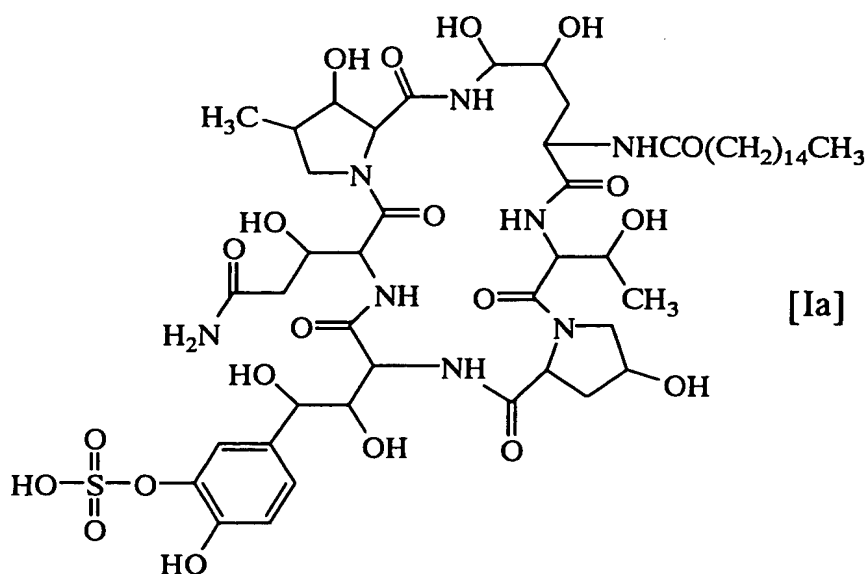
lipopeptide substance into contact with the cell extract to deacylate a side chain acyl group of the substance into an amino group.

When the cyclic lipopeptide substance is brought into
5 contact with cell extract, after completion of the culture, the culture liquid is centrifuged or filtrated to recover the cells, which are suspended in a suitable buffer, such as acetate buffer. By ultrasonication and the like, the cell are ruptured and centrifuged, and the obtained supernatant is used
10 as a cell extract.

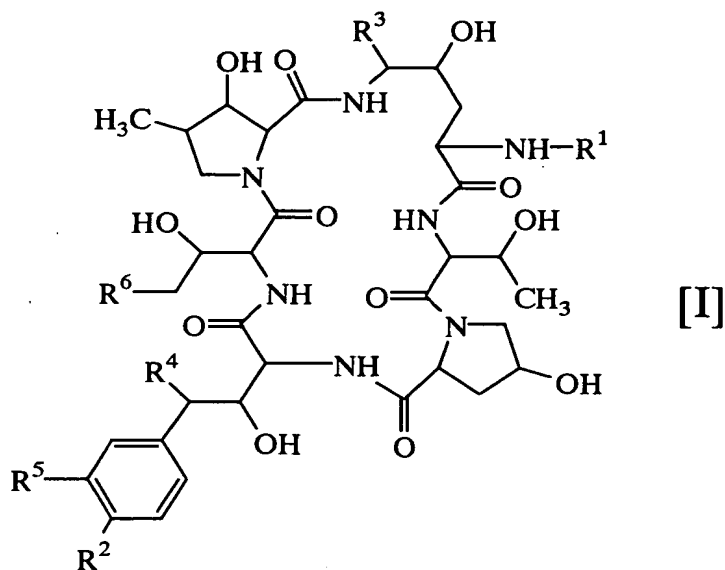
A deacylated cyclic lipopeptide substance can be also obtained by culturing a host cell producing a cyclic lipopeptide acylase in the presence of a cyclic lipopeptide substance.

15 The cyclic lipopeptide substance to be the substrate of the cyclic lipopeptide acylase of the present invention has a polypeptide ring and an "acylamino group" on the ring as a side chain. This substance may further have a different side chain. For example, the substance disclosed in WO97/32975 is
20 mentioned.

The FR901379 substance which is one example of the "cyclic lipopeptide substance" is a known substance (JP-A-3-184921) having an antifungal activity, which is produced by a
25 *microorganism* *Coleophoma* sp. F-11899 strain (FERM BP-2635; deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan, date of original deposit: October 26, 1988), and is a compound represented by the
30 following structural formula [Ia]:



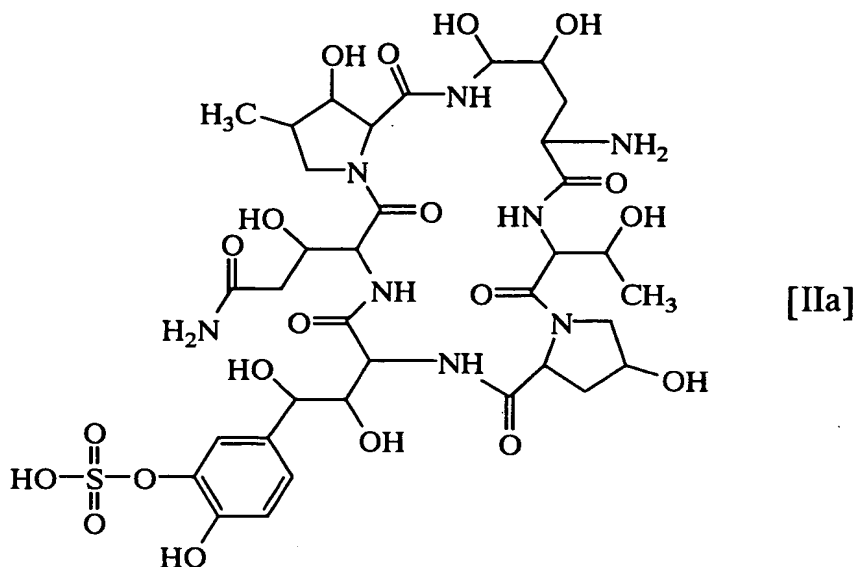
The FR901379 substance analog refers to a compound of the following formula [I] or a salt thereof.



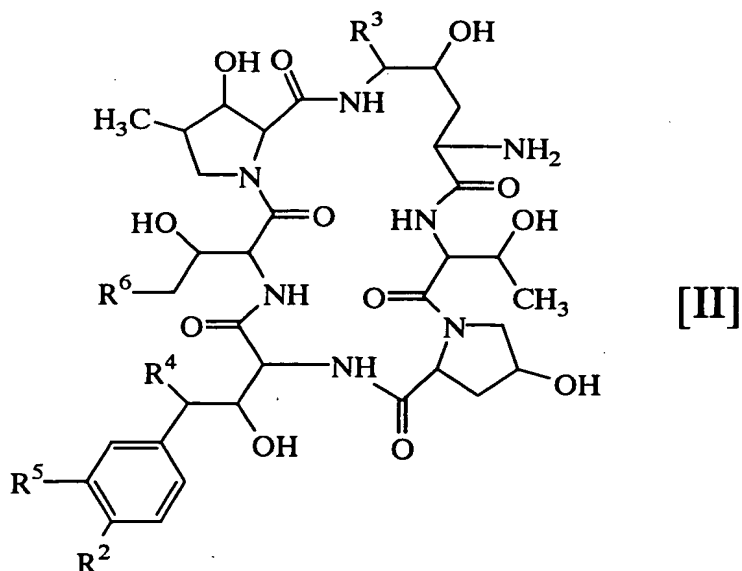
5 wherein R^1 is acyl group, R^2 is hydroxyl group or acyloxy group, R^3 is hydrogen or hydroxyl group, R^4 is hydrogen or hydroxyl group, R^5 is hydrogen or hydroxysulfonyloxy group and R^6 is hydrogen or carbamoyl group.

The cyclic lipopeptide acylase of the present invention
 10 deacylates a side chain "acylamino group" of a cyclic lipopeptide substance to lead to an "amino group". Specifically, it is an acylase that deacylates a palmitoyl side chain of FR901379 substance or a salt thereof, or an acyl

side chain of an analog of acylase FR901379 substance analog of the aforementioned formula [I], inclusive of FR901379 substance, or a salt thereof to produce a cyclic lipopeptide substance, specifically a compound (FR179642 substance) of the following structural formula [IIa] or a salt thereof



or an FR179642 analog of the following formula [II], inclusive of an FR179642 substance, or a salt thereof



wherein R², R³, R⁴, R⁵ and R⁶ mean the same groups as defined above.

Preferable salts of the compound [I] and [II] are

conventional non-toxic mono or di salts, which are exemplified by metal salt, such as alkali metal salt (e.g., sodium salt, potassium salt etc.), alkaline earth metal salt (e.g., calcium salt, magnesium salt etc.), ammonium salt, salts with organic
5 base (e.g., trimethylamine salt, triethylamine salt, pyridine salt, picoline salt, dicyclohexylamine salt, N,N'-dibenzylethylenediamine salt etc.) and the like, organic acid addition salt (e.g., formate, acetate, trifluoroacetate, maleate, tartrate, methanesulfonate, benzenesulfonate,
10 toluenesulfonate etc.), inorganic acid addition salt (e.g., hydrochloride, hydrobromate, hydroiodide, sulfate, phosphate etc.), salts with amino acid (e.g., arginine, aspartic acid, glutamic acid etc.), and the like.

After the completion of the deacylation, the deacylated
15 cyclic lipopeptide substance, specifically, an FR179642 analog (inclusive of FR179642 substance) of the formula [II] can be separated and purified from the reaction mixture by a conventionally known separation and purification methods, such as vacuum concentration, lyophilization, extraction, pH
20 adjustment, absorption resin, ion exchange resin, crystallization, recrystallization and the like, which are combined as appropriate.

Examples

The present invention is explained in detail in the
25 following by way of Examples. It is needless to say that the present invention is not limited by these Examples.

Many techniques, reactions and analysis methods used in the present invention are known per se to those of ordinary skill in the art. Unless particularly specified, the enzymes,
30 plasmids, hosts and the like are commercially available.

Example 1 Cloning of cyclic lipopeptide acylase (FR901379 acylase) produced by *Streptomyces* sp. No.6907 strain

(1) Preparation of chromosomal DNA of *Streptomyces* sp. No.6907 strain

A spoonful of a cryopreserved solution of *Streptomyces* sp. No. 6907 strain (FERM BP-5809; WO97/32975; deposited at the Fermentation Research Institute, Agency of Industrial Science and Technology, the Ministry of International Trade and Industry, 1-3, Higashi 1-chome, Tsukuba-shi, Ibaraki-ken, Japan, date of original deposit March 8, 1996, date of international deposit (transfer) February 3, 1997) was cultured in a medium (pH 6.5) containing 0.3% yeast extract, 0.5% peptone, 0.3% malt infusion, 1% glucose, 5% sucrose, 5 mM MgCl₂ and 0.5% glycine at 30°C for 48 h. The culture solution (10 ml) was centrifuged (5,000 rpm, 10 min) to collect the cells. The cells were treated using QIAGEN Genomic tip 20/G (QIAGEN Inc.) according to the protocol to give chromosomal DNA.

(2) Analysis of small subunit

(i) Design of primer for PCR from known amino terminal amino acid sequence of small subunit

The following forward primer (SF3) and reverse primer (SR2) were designed from the amino terminal amino acid sequence of FR901379 acylase small subunit

Gly Ser Gly Leu Ser Ala Val Ile Arg Tyr Thr Glu Tyr Gly Ile
Pro His His Val Ala (SEQ ID No. 5) (WO97/32975 publication)
[Note that the amino terminal amino acid sequence of this small subunit was analyzed in detail later and the accurate sequence was found to be Gly Ser Gly Leu Ser Ala Val Ile Arg Tyr Thr Glu Tyr Gly Ile Pro His Ile Val Ala (SEQ ID No. 4).].

SF3 -CTS TCS GCS GTS ATC (SEQ ID No. 6)
SR2 -GTG GTG SGG GAT SCC (SEQ ID No. 7)
S: C or G

(ii) PCR using a primer designed from known amino terminal amino acid sequence of small subunit

PCR was performed using chromosomal DNA (100 ng) of *Streptomyces* sp. No.6907 strain prepared in the above-mentioned (1) and each primer (1 nmol) designed in the above-

mentioned (i) in GeneAmp PCR System Model 2400 (Perkin-Elmer, Inc.). The reaction mixture (50 µl [in PCR buffer, 0.2 mM each dNTPs and KOD Dash (Toyobo Co., Ltd.), 1.5 unit] was subjected to 30 cycles of PCR consisting of 20 seconds of denaturing at
5 98°C, 2 seconds of annealing at 60°C, and 10 seconds of polymerization at 74°C. After amplification, a fragment (45 bp) encoding a part of the known amino terminal of a small subunit was isolated by 5% agarose gel electrophoresis.

(iii) Cloning of PCR fragment

10 The PCR amplified fragment (45 bp) isolated in the above-mentioned (ii) was treated using a pCR-Script Amp Cloning Kit (Stratagene) according to the protocol to give plasmid p3S4, in which the fragment was inserted into pCR-Script Amp SK(+).

(iv) Analysis of nucleotide sequence

15 The nucleotide sequence of plasmid p3S4 obtained in the above-mentioned (iii) was analyzed with a 310 DNA sequencer (Perkin-Elmer, Inc.) using a reverse primer (New England Biolabs Inc.), which is M13 sequencing primer, according to the dideoxy termination method according to the attached
20 protocol for sequencing. The results are shown in Fig. 1.

(3) Analysis of large subunit

(i) Design of primer for PCR from known amino terminal amino acid sequence of large subunit

The following forward primer (LF2) and reverse primer
25 (LR) were designed from the amino terminal amino acid sequence of FR901379 acylase large subunit

Ser Asn Ala Val Ala Phe Asp Gly Ser Thr Thr Val Asn Gly Arg
Gly Leu Leu Leu Gly (SEQ ID No. 3) (WO97/32975 publication)

LF2 - CS GTS GCS TTC GAC GG (SEQ ID No. 8)

30 LR - SCC SAG SAG SAG SCC (SEQ ID No. 9)

S: C or G.

(ii) PCR using a primer designed from known amino terminal amino acid sequence of large subunit

PCR was performed using chromosomal DNA (100 ng) of

Streptomyces sp. No.6907 strain prepared in the above-mentioned (1) and each primer (1 nmol) designed in the above-mentioned (3)(i) in GeneAmp PCR System Model 2400 (Perkin-Elmer, Inc.). The reaction mixture (50 µl [in PCR buffer, 0.2 mM each dNTPs and KOD Dash (Toyobo Co., Ltd.), 1.5 unit] was subjected to 30 cycles of PCR consisting of 20 seconds of denaturing at 98°C, 2 seconds of annealing at 65°C, and 10 seconds of polymerization at 74°C. After amplification, a fragment (53 bp) encoding a part of the known amino terminal of a large subunit was isolated by 5% agarose gel electrophoresis.

(iii) Cloning of PCR fragment

The PCR amplified fragment (53 bp) isolated in the above-mentioned (3)(ii) was treated using a pCR-Script Amp Cloning Kit (Stratagene) according to the protocol to give plasmid p3L1 in which the fragment was inserted into pCR-Script Amp SK(+).

(iv) Analysis of nucleotide sequence

The nucleotide sequence of plasmid p3L1 was analyzed with a 310 DNA sequencer (Perkin-Elmer, Inc.) using a reverse primer (New England Biolabs), which is M13 sequencing primer, according to the dideoxy termination method according to the attached protocol for sequencing. The results are shown in Fig. 2.

(4) Analysis of FR901379 acylase

(i) Design of primer for PCR between small and large subunits

In the acylases heretofore known, most of them are encoded in the order of a small subunit and a large subunit. Therefore, a forward primer 20S was designed based on the nucleotide sequence determined by PCR in the known amino acid sequence of the small subunit. Similarly, a reverse primer 19L was designed based on the nucleotide sequence determined by PCR in the known amino acid sequence of the large subunit.

20S -ATC CGG TAC ACG GAG TAC GG (SEQ ID No. 10)

19L -C GTT CAC CGT CGT GGA GCC (SEQ ID No. 11)

(ii) PCR between small and large subunits

PCR was performed using chromosomal DNA (100 ng) of *Streptomyces* sp. No. 6907 strain prepared in the above-mentioned (1) and each primer (20 pmol) designed in the above-mentioned (4)(i) in GeneAmp PCR System Model 2400 (Perkin-Elmer, Inc.). The reaction mixture (50 µl [in PCR buffer, 0.2 mM each dNTPs and KOD Dash (Toyobo Co., Ltd.), 2.5 units] was subjected to 30 cycles of PCR consisting of 20 seconds of denaturing at 98°C, 2 seconds of annealing at 70°C, and 20 seconds of polymerization at 74°C. After amplification, a fragment (about 600 bp) encoding a part of the acylase was isolated by 2% agarose gel electrophoresis.

(iii) Cloning of PCR fragment

The PCR amplified fragment (about 600 bp) isolated in the above-mentioned (4)(ii) was treated using a pCR-Script Amp Cloning Kit (Stratagene) according to the protocol to give plasmid pSL1 in which the fragment was inserted into pCR-Script Amp SK(+).

(iv) Analysis of nucleotide sequence

The nucleotide sequence of plasmid pSL1 obtained in the above-mentioned (4)(iii) was analyzed with a 310 DNA sequencer (Perkin-Elmer, Inc.) using a forward primer and a reverse primer (New England Biolabs Inc.), which are M13 sequencing primers, according to the dideoxy termination method according to the attached protocol for sequencing. As a result, a part of the gene seemingly the acylase was obtained.

(5) Preparation of chromosomal DNA library

The chromosomal DNA (1 µg) of *Streptomyces* sp. No. 6907 strain prepared in the above-mentioned (1) was treated with Sau3A I (100 mU) at 37°C for 10 min for partial digestion. The cosmid pcos6EMBL (1 µg, see Gene, 57, 229-237 (1987)) was treated with BamH I 5U at 37°C for 1 h. The both treated solutions were subjected to ethanol precipitation and

dissolved in 2-fold diluted TE (5 μ l, 5 mM Tris-HCl (pH 8.0), 0.5 mM), to which 10 \times T4 DNA ligase buffer (0.7 μ l, 660 mM Tris-HCl (pH 7.6), 66 mM MgCl₂, 100 mM DTT, 1 mM ATP) and T4 DNA ligase (0.7 μ l) were added. The mixture was incubated at
5 22°C for 3 h. This ligation solution (3 μ l) was subjected to in vitro packaging using GIGAPACK III XL Packaging Extract (Stratagene) according to the protocol. This packaging solution was brought into contact with an indicator strain *E. coli* XL-1 Blue MRA to construct the cosmid library.

10 (6) Screening by colony direct PCR

The cosmid clones (480 clones) obtained above were subjected to colony direct PCR using primer 20S and 19L (each 20 pmol) and GeneAmp PCR System Model 2400 (Perkin-Elmer, Inc.). The reaction mixture (20 μ l [in PCR buffer, 0.2 mM each
15 dNTPs and KOD Dash (Toyobo Co., Ltd.), 2.5 units] was subjected to 30 cycles of PCR consisting of 20 seconds of denaturing at 98°C, 2 seconds of annealing at 68°C, and 20 seconds of polymerization at 74°C. As a result, a cosmid clone No. 133 wherein about 600 bp fragment was specifically
20 amplified was obtained.

(7) Subcloning of cosmid clone No. 133

The cosmid clone No. 133 was digested with EcoR I and Pst I and the obtained about 8 kb fragment was inserted into the EcoR I/Pst I site of pUC18 to give plasmid pEP1. The plasmid
25 pEP1 was digested with EcoR I and BamH I and the obtained about 5.5 kb fragment was inserted into the EcoR I/BamH I site of pUC18 to give plasmid pEB.

(8) Nucleotide sequence analysis

The nucleotide sequence of plasmid pEB was analyzed with
30 a 310 DNA sequencer (Perkin-Elmer, Inc.) using a forward primer and a reverse primer (New England Biolabs Inc.), which are M13 sequencing primers, and synthetic oligonucleotide shown in Table 1, according to the dideoxy termination method according to the attached protocol for sequencing.

Table 1

AC1	CAA CTG CGC GTA GTC C	(SEQ ID No.13)
AC2	CAT GGG TTC CAA CGC G	(SEQ ID No.14)
AC3	GCT GTC AAC CGT CTG G	(SEQ ID No.15)
AC4	ACG CGC TGA ACG ATC C	(SEQ ID No.16)
AC5	CGG ACC TGG ACC TAC C	(SEQ ID No.17)
AC6	GTG GGT GAA CAC GAT CG	(SEQ ID No.18)
AC7	GAC CTT CAG CGG CAG C	(SEQ ID No.19)
AC8	CAA GTG GTG TGC GGC G	(SEQ ID No.20)
AC9	GTC GCT GGG CAT CTG G	(SEQ ID No.21)
AC10	GCT GCT GAC GTA CTC C	(SEQ ID No.22)
AC11	GTC AAC CGC ATG GTC C	(SEQ ID No.23)
AC12	ATC GCC TGG ATC GTC G	(SEQ ID No.24)
AC13	CGT CAG CGC GAT CAC C	(SEQ ID No.25)
AC14	GGT GTA CAG CAG CTG C	(SEQ ID No.26)
AC15	CTC CCT CGT CCT GAC C	(SEQ ID No.27)
AC16	GAG TTG TGC GCG TAG G	(SEQ ID No.28)
AC17	TGA CGC TTG GCC GTC C	(SEQ ID No.29)
AC18	GAC TAC GCG CAG TTG G	(SEQ ID No.30)
AC19	TAC AAC GCG TGG ATC G	(SEQ ID No.31)
AC20	GGT GAT CCG GTT CTG C	(SEQ ID No.32)
AC21	GGG TAG TGC GGG TTG C	(SEQ ID No.33)
AC22	CTG CAT CAG CTC AGC C	(SEQ ID No.34)
AC23	GTC CAC CAC TGG GTG C	(SEQ ID No.35)
AC24	GAA GCG GGG TAG GTG G	(SEQ ID No.36)
AC25	CCG GTG CTG AAG AAC C	(SEQ ID No.37)
AC26	CTG CCG CTG AAG GTC C	(SEQ ID No.38)
AC27	TCG AAC GGC GTC CTC C	(SEQ ID No.39)
AC28	TGG AGG ACG CCG TTC G	(SEQ ID No.40)
AC29	GCC TGG ATG TAG CTG G	(SEQ ID No.41)
AC30	GGA CAT CGC GCG TTC G	(SEQ ID No.42)
AC31	CGA ACG CGC GAT GTC C	(SEQ ID No.43)
AC32	CCG TGA CCA TGC GTG C	(SEQ ID No.44)
AC33	GCA CGC ATG GTC ACG G	(SEQ ID No.45)
AC34	GAG GAG ACC TAC CTC G	(SEQ ID No.46)
AC35	AGG TCC CGC TAC GAC G	(SEQ ID No.47)
AC36	GAC CAT GCG GTT GAC G	(SEQ ID No.48)
AC37	CAG TTC CGC CTC GTC G	(SEQ ID No.49)
AC38	CAG GTG GAC GTT GTC G	(SEQ ID No.50)
AC39	GTC GCT GAC GAT CAC G	(SEQ ID No.51)
AC40	GTG ATC GTC AGC GAC C	(SEQ ID No.52)
AC41	GGC GGT GAT GAA GTC G	(SEQ ID No.53)
AC42	CGA CTT CAT CAC CGC C	(SEQ ID No.54)
AC43	GGC GAC TTC TTC ACC G	(SEQ ID No.55)
AC44	CGG TGA AGA AGT CGC C	(SEQ ID No.56)
AC45	CCA GAC GGT TGA CAG C	(SEQ ID No.57)

From the analysis of the nucleotide sequence, ORF was found, in which DNA sequences corresponding to the amino terminal amino acid sequences of a small subunit and a large subunit were included entirely. The nucleotide sequence of
5 plasmid pEB containing the acylase gene is shown in SEQ ID No. 1.

Example 2 Expression of cyclic lipopeptide acylase in a host cell

(1) Construction of *Streptomyces lividans* 1326/pIJ702-SB

10 Plasmid pSB wherein EcoR I site of plasmid pEB was changed to Sac I site was to be constructed. First, a synthetic oligomer (100 pmol, AAT TGA GCT C; SEQ ID No. 12) was treated with 30U T4 polynucleotide kinase at 37°C for 1 h to phosphorylate the 5'-OH terminal. The reaction mixture was
15 heated at 70°C for 10 min to inactivate the T4 polynucleotide kinase. Separately, 1 µg of plasmid pEB was treated with 5U EcoR I and with 1U Bacterial Alkaline Phosphatase (BAP) at 37°C for 1 h to dephosphorylate the 5' terminal. These two treatment solutions were ligated with Ligation High (Toyobo
20 Co., Ltd.) to construct plasmid pSB.

The plasmid pSB (5 µg) was treated with 20 U Sac I and 20U BamH I to give a 5.7 kb Sac I-BamH I fragment containing an acylase gene. pIJ702 (2 µg) (ATCC 35287), which is a vector for *Actinomycetes*, was treated with 10 U Sac I and 10 U Bgl II
25 and ligated with 5.7 kb Sac I-BamH I fragment containing an acylase gene prepared beforehand in the presence of Ligation High (Toyobo Co., Ltd.). The ligation solution was used to transform *Streptomyces lividans* 1326 strain (J. General Microbiology 1983, 129, 2703-2713) according to the method
30 described in "Genetic Manipulation of *Streptomyces*. A Laboratory Manual. The John Innes Foundation, Norwich, UK, 1985". Of the obtained transformed strains, one strain was taken as *Streptomyces lividans* 1326/pIJ702-SB (Fig. 3).

(2) Culture of the transformant and expression of FR901379

acylase

A medium (10 ml) containing 5% sucrose, 1% glucose, 0.3% yeast extract (Difco), 0.5% bactopectone (Difco), 0.3% meat extract (Difco), 5 mM MgCl₂, 0.5% glycine and 50 µg/ml of
5 thiostrepton (pH 6.5) was placed in a 100 ml Erlenmeyer flask, in which the bacterial cells (5 mm square) of the transformed strain *Streptomyces lividans* 1326/pIJ702-SB were inoculated. The cells were cultured at 30°C for 3 days (260 rpm) and the FR901379 acylase activity of the culture solution was
10 determined. As a result, an activity to produce 30 mg of FR179642 (deacylated FR901379 substance) per 1 ml of the culture solution per 1 h was found.

The culture time until the acylase activity reached the maximum was shortened to 2 - 3 days for transformed strain
15 *Streptomyces lividans* 1326/pIJ702-SB. Since it was 7 days for *Streptomyces* sp. No. 6907, the time was shorter than half.

The acylase activity was determined as follows.

<Measurement of acylase activity>

The culture solution (0.1 ml) was added to a solution
20 consisting of 100 mg/ml aqueous FR901379 (see WO97/32975) solution (0.1 ml), phosphate buffer (pH 6.0, 0.1 ml), methanol (0.1 ml) and distilled water (0.6 ml) and reacted at 37°C (125 rpm). After 15 min, 4% acetic acid (1 ml) and distilled water (2 ml) were added to terminate the reaction. The produced
25 FR179642 (deacylated FR901379 substance) was quantitatively determined by high performance liquid chromatography (HPLC) under the following conditions.

column; Kaseisorb LC PO Super (4.6 mm I.D. × 250 mm) (Tokyo Kasei Kogyo Co., Ltd)

30 column temperature; 50°C

eluate; distilled water:methanol:phosphoric acid = 960:40:1
flow rate; 1 ml/min

detection; UV-215 nm

Example 3

(1) Preparation of fermented solution

The PM-1 medium (50 mL) (6% Nisshoku #3600, 3% defatted soybean meal powder, 0.5% CaCO_3 , 0.005% thiostrepton, pH non-adjusted) containing thiostrepton (50 $\mu\text{g/mL}$) was inoculated in
5 a 500 mL flask, and after inoculating bacterial cells (5 mm square) of the transformed strain *Streptomyces lividans* 1326/pIJ702-SB, the cells were cultured at 30°C for 3 days. The culture (2.5 mL) was inoculated in a 500 mL flask containing 50 mL of SG medium (8% maltose, 3% defatted soybean
10 meal powder, 3% defatted wheat germ, 0.5% CaCO_3 , 0.005% thiostrepton, pH non-adjusted) and cultured at 30°C for 3 days.
(2) Purification of FR901379 acylase

4M KCl (8 mL) was added to the fermented solution (24 mL) and the mixture was left standing overnight at 4°C, which was
15 followed by centrifugation (10,000 rpm, 10 min) to give a supernatant and used as a KCl extract solution. This KCl extract solution was concentrated 10-fold with Microcon 50 (Millipore Corporation) and returned to the original liquid amount with 0.5M sodium phosphate buffer (pH 6.0). This step
20 was repeated twice to remove low molecular weight protein.

(3) SDS-PAGE analysis

SDS-PAGE analysis was performed using multigel 10/20 or 15/25 (Daiichi Pure Chemicals Co., Ltd.; acrylamide 10-20% or 15-25% gradient gel).

25 (4) Measurement of FR901379 acylase activity

Measured according to Example 2.

(5) Quantification of protein

Measured by DC Protein Assay (Lowry method; Biolad) using albumin as a standard.

30 (6) Amino terminal amino acid sequence analysis

The protein in a gel after SDS-PAGE was transferred to PVDF membrane like electrophoresis by Horizblot (Atto Co. Ltd.) and stained with CBB. The objective band was cleaved with scissors and subjected to amino terminal amino acid

sequence analysis.

From the analysis results, it was found that 45% of the cyclic lipopeptide acylase small subunit had an N-terminal amino acid sequence described in SEQ ID No. 4 and 55% thereof had an N-terminal amino acid sequence wherein a serine residue was added to the N terminal thereof.

Industrial Applicability

The recombinant cyclic lipopeptide acylase of the present invention, which is obtained from a transformant obtained by introducing a gene encoding cyclic lipopeptide acylase, has the same level of activity as does an acylase obtained by culturing a natural separation strain that produces cyclic lipopeptide acylase such as conventional *Streptomyces* sp. No. 6907 strain, *Streptomyces anulatus* No. 8703 strain and the like, and the use of the transformant has made it possible to shorten the time necessary for culture (time necessary for producing cyclic lipopeptide acylase).

This application is based on patent application No. 189644/1999 filed in Japan, the contents of which are hereby incorporated by reference.

Sequence Listing Free Text

SEQ ID NO: 6 Oligonucleotide designed to act as PCR primer (forward) to amplify the DNA coding N-terminal amino acid sequences of FR901379 acyrase small subunit.

SEQ ID NO: 7 Oligonucleotide designed to act as PCR primer (reverse) to amplify the DNA coding N-terminal amino acid sequences of FR901379 acyrase small subunit.

SEQ ID NO: 8 Oligonucleotide designed to act as PCR primer (forward) to amplify the DNA coding N-terminal amino acid sequences of FR901379 acyrase large subunit.

SEQ ID NO: 9 Oligonucleotide designed to act as PCR primer

(reverse) to amplify the DNA coding N-terminal amino acid sequences of FR901379 acyrase large subunit.

SEQ ID NO: 10 Oligonucleotide designed to act as PCR primer (forward) to amplify the DNA coding amino acids between
5 FR901379 acyrase small subunit and large subunit.

SEQ ID NO: 11 Oligonucleotide designed to act as PCR primer (reverse) to amplify the DNA coding amino acids between FR901379 acyrase small subunit and large subunit.

SEQ ID NO: 12 Oligonucleotide designed for use in changing
10 restriction site from EcoR I site to Sac I site.

SEQ ID NO: 13 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 14 Oligonucleotide designed to act as sequencing primer.

15 SEQ ID NO: 15 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 16 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 17 Oligonucleotide designed to act as sequencing
20 primer.

SEQ ID NO: 18 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 19 Oligonucleotide designed to act as sequencing primer.

25 SEQ ID NO: 20 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 21 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 22 Oligonucleotide designed to act as sequencing
30 primer.

SEQ ID NO: 23 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 24 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 25 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 26 Oligonucleotide designed to act as sequencing primer.

5 SEQ ID NO: 27 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 28 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 29 Oligonucleotide designed to act as sequencing
10 primer.

SEQ ID NO: 30 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 31 Oligonucleotide designed to act as sequencing primer.

15 SEQ ID NO: 32 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 33 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 34 Oligonucleotide designed to act as sequencing
20 primer.

SEQ ID NO: 35 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 36 Oligonucleotide designed to act as sequencing primer.

25 SEQ ID NO: 37 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 38 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 39 Oligonucleotide designed to act as sequencing
30 primer.

SEQ ID NO: 40 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 41 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 42 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 43 Oligonucleotide designed to act as sequencing primer.

5 SEQ ID NO: 44 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 45 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 46 Oligonucleotide designed to act as sequencing
10 primer.

SEQ ID NO: 47 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 48 Oligonucleotide designed to act as sequencing primer.

15 SEQ ID NO: 49 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 50 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 51 Oligonucleotide designed to act as sequencing
20 primer.

SEQ ID NO: 52 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 53 Oligonucleotide designed to act as sequencing primer.

25 SEQ ID NO: 54 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 55 Oligonucleotide designed to act as sequencing primer.

SEQ ID NO: 56 Oligonucleotide designed to act as sequencing
30 primer.

SEQ ID NO: 57 Oligonucleotide designed to act as sequencing primer.